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FACTORS INFLUENCING THE REGRESSION OF CORPORA LUTEA IN THE EWE, RABBIT AND RAT¹

H. G. Spies, Kansas Agricultural Experiment Station, Manhattan

Stages in the life of corpora lutea may be divided logically into formation, function and regression. Mechanisms controlling each stage may be independent or overlapping depending on the species. Anderson *et al.*, (4) have pointed to lack of current evidence to allow a clear distinction between luteinizing hormone (LH) and luteotropic hormone (LTH) in farm animals. Initial LTH release is probably sufficient to form and maintain the corpora lutea for the normal estrous cycle (Aldred *et al.*, 1). Maintenance of corpora lutea for the normal duration of an estrous cycle in hypophysectomized ewes (Denamur and Mauleon, 6) and sows (du Mesnil du Buisson and Léglise, 19) supports this theory. Nalbandov (20) has suggested a second release of luteotropin is initiated due to mechanics of placentation.

Factors responsible for regression of the corpora lutea in most species have not been determined. However, the observed prolongation of functional corpora lutea in several species (review article, Anderson *et al.* 3) following hysterectomy implicate the uterus. Postulates include a uterine stimulus that blocks LTH release, a uterine provoked luteolytic factor (LLF), or a combination of both.

Ewe

Hysterectomy extends the life of the corpus luteum of the cycling ewe to approximately 160 days (Kiracofe and Spies, 14). The mechanism causing regression of corpora lutea in the hysterectomized ewe appears to differ from the mechanism of cycling ewes. Corpora lutea induced following natural ovulation in the hysterectomized ewe do not regress synchronously with naturally formed corpora lutea as reported for the cycling ewe (Inskeep *et al.*, 10), but persist for about 160 days from induction (Kiracofe and Spies, unpublished manuscripts). Asynchronous regression of induced and naturally formed corpora lutea in hysterectomized ewes may indicate the presence of a LLF in the intact ewe. The necessity of the pituitary to maintain corpora lutea in hysterectomized ewes (Denamur and Mauleon, 6) beyond 20 days suggests that asynchronous regression of induced and naturally formed corpora lutea may result from a difference in competence of different-aged luteal tissues to gonadotropin stimulation. Differences in response of sheep luteal tissue to a given level of PMS in vitro support this hypothesis (Legault-Demare *et al.*, 16).

Thus far attempts to isolate a uterine LLF have failed. Ether extracts of uteri removed from ewes during estrus to 4 to 7 days post estrus did not influence the weight or histology of the corpora lutea when injected into hysterectomized ewes during 11 - 19 days following ovulation (Kiracofe *et al.*, 14). Freeze-dried extracts of uteri removed on days 12 to 14 following ovulation or on the day of estrus have also failed to regress the corpora lutea when injected into cyclic or pregnant ewes hysterectomized prior to injections, or when injected into normal 25-day pregnant ewes (Kiracofe and Spies, unpublished manuscript). These results must be used cautiously as extraction procedure; amount of extract or type of test animal may be involved in the negative response. A small percentage of hysterectomized ewes receiving uterine extracts possessed small accessory corpora lutea at slaughter. None was observed at preinjection laparotomy or in noninjected hysterectomized controls. It is not known if gonadotropin stimulation resulted from uterine extracts.

Bilateral ligation of the mid-uterine arteries and veins prolonged the life of cyclic corpora lutea in ewes, while unilateral ligations had no effect (Kiracofe *et al.*, 13). A species difference may exist between ewes, sows and guinea pigs (Rowlands, 24) in regard to a localized effect of the uterus on the corpora lutea (du Mesnil du Buisson, 18). The uterine effect on the luteal regression mechanism in the ewe probably occurs rapidly. Stormshak *et al.* (25) indicated that a decrease in corpora lutea weight and progesterone concentration had not occurred by day 14 in the cycling ewe. Also, hysterectomy of ewes as late as day 16 post ovulation resulted in maintenance of the corpora lutea (Kiracofe and Spies, 15). This agrees with results in the guinea pig where hysterectomy on day 15 of the cycle maintained the corpora lutea (Rowland, 23). The mechanism appears to act somewhat earlier in the pig since hysterectomy after days 16 - 18 of the 21-day cycle resulted in regression of the corpora lutea and new ovulation (Andersson *et al.*, 2). In addition du Mesnil du Buisson (18) reported that unilateral regression of corpora lutea in the pig was evoked between day 14 and 16 post ovulation. Although corpora lutea are maintained in ewes hysterectomized before day 16 of the cycle, the corpora lutea are smaller when the uterus is removed after day 14 (Kiracofe and Spies, 15). A slower rate of luteal regression has been reported in hypophysectomized rats than in intact animals.

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(Greep, 8). Also du Mesnil du Buisson and Léglise (19) observed decreased luteal regression in hypophysectomized, hysterectomized sows compared with intact females, suggesting a possible uterine-pituitary interaction.

Rabbit

Induced corpora lutea of the cycling gilt regress asynchronously (Neill and Day, 21) as do corpora lutea of hysterectomized ewes, in contrast to the synchronous regression of corpora lutea in cycling ewes (Inskeep *et al.*, 10). Rabbits differ from both ewes and pigs as induction of a second group of corpora lutea in pituitary intact pseudopregnant rabbits will cause rapid regression of the initial group of corpora lutea at any stage of pseudopregnancy beyond day 4 (Coon and Spies, 5). HCG, NIH-LH or NIH-FSH plus LH produced new ovulations and involution of the initial set of corpora lutea. Four-day-old corpora lutea did not regress when does were treated with HCG, but histologically appeared small, avascular, and poorly developed. Corpora lutea induced at day 3 of pseudopregnancy and initial corpora lutea regress synchronously; however, regression occurred approximately 18 days after the second ovulation. These results differ from those reported for the cycling ewe.

Estrogen was reported to be luteotropic in the rabbit (Robson, 22; Hammond Jr. and Robson, 9). LH treatment after day 4 post ovulation in the pituitary intact rabbit appears to block estrogen, thus causing corpora lutea regression. Estrone administered concurrently with LH prevents regression of the initially formed corpora lutea (Coon and Spies, 5). A second ovulation may be provoked and the two groups of corpora lutea regress asynchronously about day 18 post respective ovulations. Observations on corpora lutea induced in the hysterectomized-pseudo-pregnant rabbit are similar to observed results in the uterine-intact rabbit except the second group of corpora lutea persists 25-28 days (Spies and Coon, unpublished manuscript). Kilpatrick *et al.* (12) reported that LH was luteotropic in hypophysectomized does. The contrasting effect of LH in pituitary intact does, compared with hypophysectomized does, suggests LH may block estrogen via a pituitary pathway.

Rat

verett (7) has suggested that the same neurohumoral factors from the hypothalamus that stimulate LH discharge inhibit prolactin release in the rat. Greep (8) has suggested LH has a luteolytic effect in the hypophysectomized rat, and although this work has not been confirmed, de Jongh and Wolthuis (11) indicated Rothchild has recently observed that exogenous LH injected into rats with an extra pituitary

placed in the renal capsule caused decreased size and progesterone production. Melampy *et al.* (17) has suggested their work with hysterectomized-pseudopregnant rats provided evidence of an uterine-luteolytic mechanism. Spies and Kiracofe (unpublished manuscript) have not been able to identify a uterine LLF from rat uterine extracts or parabiotic rats. Freeze-dried uterine extracts from estrual rats did not shorten the vaginal cycle of hysterectomized-pseudopregnant rats. Parabiotic union of ovariectomized females with intact-pseudopregnant or hysterectomized-pseudopregnant rats increased vaginal cornification of the ovarian intact partner, probably as a result of increased estrogen output. However, the presence of India ink-marked, histologically functional corpora lutea suggests the life of the corpora lutea was not shortened. Vaginal cycles of normal intact cycling females were not affected when paired with hysterectomized-pseudopregnant females, nor were any effects on ovarian histology of the hysterectomized-pseudopregnant partner observed.

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DISCUSSION

DR. SPIES: I would like to have a definition of the term "luteolytic factor." Are we simply talking about a "factor" which blocks LTH or does the "factor" cause regression of the corpus luteum in the presence of luteotropin?

DR. MELAMPY: Dr. Day, do you wish to answer this question?

DR. DAY: I don't believe I would call it luteolytic. Maybe use of the term has been confined to a direct effect. I think it could be some factor which is anti-luteotropic but whether this is gonadotropic or not I do not know. But it could act, let us say, through the pituitary.

DR. SPIES: I wonder if we are all consistent in the use of this term. Are we all using it in this light or do we have differences in the way different workers are using the term as results are reported in the literature?

DR. MELAMPY: It is realized that there are many unsolved problems in the field of corpus luteum physiology. This is particularly true as experimental results become available from investigations dealing with different species. Among these problems are what physiologic factor(s) are involved in the formation of the corpus luteum? Is this associated with LH activity or is it dependent upon a specific luteotropin? Also, what mechanisms are involved in the maintenance of progesterone secretion? What are the functional and morphologic changes associated with the initiation of luteal regression? What is the role of the uterus in luteal function? The effect of hysterectomy on ovarian function has been studied in several species. Total hysterectomy has been observed to cause persistence of corpora lutea for a period of time approaching or exceeding the length of gestation in the guinea pig, ewe, sow, and cow, but hysterectomy of the ferret and opossum has no apparent effect on ovarian function or mating behavior. The effects observed following uterus removal in the monkey and woman are not well defined. This may be due to a lack of systematically controlled observations over a period of time. Hysterectomy in the unmated rat and rabbit has no apparent immediate effect on ovarian activity, whereas hysterectomy of pregnant and pseudopregnant females of these species results in postponement of luteal regression. It is to be noted that hysterectomy leads eventually to ovarian degeneration in several species. The physiologic basis of uterine regulation of luteal persistence is still obscure. Results from experimental work on the heifer, gilt, ewe, and guinea pig indicate the absence of the uterus produces a physiologic state which is compatible with persistence of functional corpora lutea. Furthermore non-specific portions of the uterine horns are adequate to initiate the onset of luteal regression during the estrous cycle of the unmated female of these species. The occurrence of estrous

cycles has been observed in gilts and guinea pigs following uterine autotransplants. It is concluded that a functional endometrium appears to be necessary for the initiation of luteal regression in gilts and guinea pigs with intact uteri as well as in animals with autotransplanted uteri. These observations suggest that a non-neural uterine stimulus is possibly involved in regulating the life span of the corpus luteum in certain species. It is possible that the uterus of the non-pregnant female maintains a positive inhibition of pituitary luteotropin release. This may be either hormonal or neuroendocrine in nature. In pregnancy and following hysterectomy this inhibition is lacking, hence, the persistence of functional corpora lutea. It is suggested that the uterus functions as a pacemaker in determining the longevity of corpora lutea and, as a result, regulates the initiation of luteal regression and hence the occurrence of cycles.

Dr. Anderson, do you want to comment on any of these questions?

DR. ANDERSON: With regard to Dr. Spies' question about the term "luteolytic factor", I think we may refer to luteolytic action, which in the case of the uterus could be the result of a particular uterine physiologic state at a certain stage of the estrous cycle rather than attributing the luteolysis to a "factor" of uterine origin.

DR. MELAMPY: I would like to ask Dr. Nalbandov a question. What do you think about the significance of LH and progesterone production by the corpus luteum?

DR. NALBANDOV: First, may I just call your attention to the fact that the oft-quoted work of Greep which dates back to the 1930's, that LH is luteolytic in rats, has been withdrawn by Greep in the publication called "Control of Ovulation," in which he states that he has repeated this work within the last few years with more purified LH preparations and has not found it to be luteolytic. I noticed that several people have quoted his work, the earlier work, without quoting the correction which he has made in subsequent years.

In the same volume, "Control of Ovulation," some work by Parlow is quoted which was later confirmed by him, (Recent Progress in Hormone Research, 1961) that LH is luteotropic in the rat, but in an entirely different sense. If you hypophysectomize the rat and treat it with LH, then, instead of producing progesterone, the rat now produces estrogen in sufficient quantities to cause vaginal cornification which persists for prolonged periods of time. This occurs many months after hypophysectomy. These hormones are apparently potentially able to affect tissue which was initially intended to produce progesterone in such a way as to make it secrete estrogen. We have been very careful in our own work not to speak of luteotropins but of luteotropic factors,

and this is because I personally have no idea what luteotropin will turn out to be. My suspicion is that it may be LH, but it may equally well turn out to be an as yet unidentified factor. That is the best answer I can give you at the time.

DR. MELAMPY: Thank you. An important contribution by T. Eto, H. Masuda, Y. Shzuki, and T. Hosi (Japanese Journal of Animal Reproduction 8:34-40, 1962) should be mentioned at this time. These investigators determined the progesterone and pregn-4-ene-20 α -ol-3-one concentrations in rat ovarian venous blood at different stages of the reproductive cycle. It was found that progesterone concentration increases to a maximum during the afternoon of proestrus and reaches approximately 110 mcg./100 ml. Following this peak it declines but shows an increase again in early diestrus. Pregn-4-ene-20 α -ol-3-one is secreted throughout the estrous cycle and attains a maximum value of approximately 240 mcg./100 ml. of blood at early diestrus. During gestation the progesterone concentration is high on day 4 and reaches a maximum value on day 15. It is low, however, immediately before and after parturition. On the other hand pregn-4-ene-20 α -ol-3-one concentration is lower during the first half of gestation than during the estrous cycle, but at day 15 it is approximately 260 mcg./100 ml. On day 8 of lactation blood progesterone concentration is high and it is higher in females with litters of 6 pups than in those with 2. Ovaries of hypophysectomized rats bearing renal pituitary grafts secrete a large amount of progesterone but little pregn-4-ene-20 α -ol-3-one.

DR. DZIUK: Before we get on to the slide, I would like to ask two questions that may be related. (1) How does unilateral regression of the corpora lutea occur in the pig if the central nervous system is involved? And (2) Does the number of embryos influence the corpus luteum number so that we perhaps have been looking at corpora lutea counts and embryo counts all this time and assuming wrongly a certain proportion of embryonal deaths? Are we actually looking at a cause and effect in which the number of embryos may influence the number of corpora lutea present?

And now I would like to show the slide. I would like to suggest that perhaps LH, as we think of it, causes ovulation but does not necessarily cause luteinization (table 1).

These animals were treated with high levels of progesterone for nine days and then, since no further treatment is applied, wouldn't ovulate spontaneously for at least 10 days, and most of them wouldn't ovulate for about 20 days (Dziuk and Baker, 1962, Jour. Anim. Sci. 21: 697). On the 6th day after the last progesterone feeding we performed a laparotomy and punctured the follicles. All gilts were slaughtered 7 days after laparotomy. The ovulating "hormone" here was a scalpel or needle. The "C" represents a triangular cut in the follicle wall

and the "S" is a puncture with scarifications. We thought we were not getting enough bleeding by just puncturing, which is indicated by the letter "P". Actually this is a little bit reversed. We started out puncturing the follicles and letting the fluid out. It turned out, as you can see, in gilts 1 through 3, that we got about the same number of follicles at slaughter as we had at laparotomy and no corpora lutea were formed when follicles were punctured. So, we thought the follicles were healing over. Later we cut or scarified follicles on one ovary and punctured follicles on the opposite ovary in gilts 4 through 9. At autopsy we observed corpora lutea on the cut or scarified side but few corpora lutea on the ovaries whose follicles had been punctured. As it turns out, the size of the hole seemed to make a considerable difference in whether we got corpora lutea formed or not. This suggests to me, that LH causes ovulation but has little to do with corpora lutea formation, and that the corpus luteum would form if the fluid was just released from the follicle.

DR. MELAMPY: Do you want to comment on that?

DR. ANDERSON: The unilateral regression of corpora lutea in gilts on the ovary on the side with a nongravid uterine fragment has been observed under experimental conditions by du Mesnil du Buisson (Compt. Rend. Acad. Sci. 253:727, 1961) and by Rathmacher and Anderson (Jour. Anim. Sci. 22:1139, 1963). This apparent luteolytic action of the uterine fragment may be a local effect. A local humoral or neuro-humoral effect may be present from the nongravid uterine fragment on the adjacent ovary. Also, alterations in hemodynamic relationships between the uterus and ovary may contribute to this phenomenon. Gilts with uterine autotransplants exhibit normal estrous cycles which would rule out at least major neural pathways for the uterine action that results in luteal regression. However, vasomotor nerves would be present in the autotransplanted uterus.

DR. MELAMPY: Dr. Casida, would you like to comment on the triangular ovulations?

DR. CASIDA: I am going to comment on unilateral regression.

One point of view on the cause of regression of corpora at the end of the cycle, or at least at the end of pseudopregnancy in the rat, is that gonadotropins, presumably FSH and LH, eventually come into the picture and interfere with the action of luteotropin for maintenance purposes. I believe this is the point of view of Dr. Rothchild. This raises the question as to whether a substance may not be produced in the uterus which is effective locally and which plays a role with the gonadotropin in bringing about regression of corpora. Perhaps sensitizing the corpora lutea to the action of gonadotropins to bring about the regression.

It seemed to us that the rabbit might be a very good animal in which to check this point

Table 1.--Corpus luteum formation after follicle puncturing in the pig

Gilt	Left Ovary				Right Ovary			
	Laparotomy		Autopsy		Laparotomy		Autopsy	
	Follicles no.	Type of cut ¹	Corpora lutea no.	Follicles no.	Follicles no.	Type of cut	Corpora lutea no.	Follicles no.
1	9	P	0	5	5	P	0	3
2	10	P	0	9	5	P	0	2
3	6	P	0	5	9	P	0	8
4	5	S	5	1	8	P	5	1
5	9	S	6	0	11	P	7	0
6	6	S	8	0	8	P	6	0
7	4	C	3	0	9	P	0	6
8	10	C	6	0	6	P	0	8
9	6	C	6	1	10	P	0	4
10	6	C	6	0	7	S	4	1
11	8	C	5	6	6	S	0	10
12	7	C	0	1	7	S	0	8
13	11	C	11	0	7	C	7	0
14	9	C	9	0	5	C	5	0
15	12	C	12	0	5	C	8	0
16	9	C	5	5	13	C	2	6
17	12	C	6	4	4	C	4	1
18	6	C	4	2	8	C	7	2
19	7	C	4	2	8	C	4	3

¹P - Puncture follicle with suturing needle.

S - Puncture follicle and rub inside of follicle with needle.

C - Cut follicle with "V" shaped incision 4 mm. each side of V.

where the injection of LH, as Dr. Spies indicated, causes regression of the corpora lutea. If there is a hysterectomy of these animals, will it work? That is, can injected LH bring about regression of corpora lutea in the hysterectomized animals? Regression did occur in hysterectomized animals and we did not get evidence for a uterine factor which could act locally between the gonadotropins and the corpus to bring about regression.

I might raise a question regarding your statement that estrogen maintains corpora lutea, or is it luteotropic in the hypophysectomized rat? Am I quoting you correctly on that? I have been unable to substantiate this point. There is evidence, I believe, that estrogen in the hypophysectomized rat will cause a marked change in the amount of granulosa tissue and in the number of follicles that show marked hypertrophy, and it will also affect, perhaps, the ability of PMS or HCG to bring about excessive stimulation or even some luteinization, but does this mean that estrogen is luteotropic in the hypophysectomized animals? I doubt that it does. It may be a matter of definition. What do we mean by luteotropic? Does a substance which will synergize the follicle-stimulating-luteinizing action of something like HCG really qualify?

DR. MELAMPY: With regard to Dr. Casida's comment pertaining to the effect of estrogen on the rat ovary, Bradbury (Endocrinology 68:115-120, 1961) observed ovaries following application of crystalline estradiol or stilbestrol to one ovary of the immature rat leaving the other ovary untreated. It was found that the estrogen-treated ovary exhibited several unilaterally differentiated responses: (1) a greater increase in weight, (2) an increased responsiveness to endogenous and exogenous gonadotropins. In control experiments crystalline testosterone or progesterone was applied to one ovary with, or without, estradiol being placed on the other ovary. These experiments demonstrate that estrogen has a local stimulatory effect within the ovary as well as a systemic effect via the pituitary. Pencharz (Science 91:554, 1940) and Williams (Nature 145:338, 1940) observed that stilbestrol pellets implanted into rats at time of hypophysectomy not only increased ovarian weights but also made the ovaries more responsive to exogenous gonadotropins. Stilbestrol has been used in hypophysectomized and intact rats to augment ovarian response to gonadotropins by Payne and Runser (Endocrinology 65:383, 1959) and Meyer and Bradbury (Endocrinology 66:121, 1960). The physiologic basis of the observed stimulating action of estrogen on ovarian function is unknown at present.

I would like to briefly mention some preliminary observations we have made relative to the physiologic state of the uterus to luteal function in the rat. In this experiment it was found that the duration of pseudopregnancy in intact rats (Holtzman strain) is 14.4 ± 0.44 days.

This is extended to 22.1 ± 0.84 days following hysterectomy. In parabiotic rats, with one animal of each pair having an intact uterus and the other hysterectomized, the length of the pseudopregnancy in the hysterectomized rat was significantly reduced when the parabiotic union was made either before or after induction of pseudopregnancy. Homotransplantation of an estrous uterus from one animal into the abdominal cavity of its pseudopregnant littermate resulted in a significant reduction in the length of diestrus when the transplant remained viable. It was also observed that autotransplants of ovaries into the uterine horns of estrous rats reduced the duration of pseudopregnancy to 11.5 ± 0.47 days as compared with 14.4 ± 0.44 days in the control groups ($P < 0.01$).

I would like to ask Dr. Hansel if he would like to comment on LH and progesterone synthesis in luteal tissue.

DR. DONALDSON: I think that what I am supposed to say is we think LH is luteotropic in the cow. The evidence for this is based upon overcoming oxytocin inhibition with an LH preparation which does not contain any FSH and the ability to do this with HCG, but not with HCG that has been incubated with 6-M-urea to destroy the LH component. The ability of a crude pituitary preparation to overcome oxytocin inhibition and the inability of bovine prolactin or ovine prolactin to do so are shown in the data on this slide. What we did was to take Holstein heifers, treat them with oxytocin, and take the corpus luteum by surgery either on day 4 or day 7. As you can see, on day 4, oxytocin did not affect luteal function as measured by size, progesterone content per gram, or total progesterone. Oxytocin reduced the total progesterone, progesterone per gram, and the corpus luteum weight on day 7. Now, if we gave HCG on top of this on day 4, nothing happened, but at day 7 we got an increase in all of these parameters. Bovine LH did the same; prolactin did not and urea incubated HCG did not.

To support this claim, we collected pituitaries on day 0, day 4, and day 7; 10 pituitaries on each of these days, half of which were treated with oxytocin and half of which were not. The data in this slide show that if a single injection of oxytocin is given as soon as we detect an animal in heat and then she is killed six hours after she is detected in heat, the total gonadotropin content of the pituitary is halved. This supports the observation made some years ago that oxytocin given during estrus will hasten ovulation.

At day 4 oxytocin did nothing to the pituitary gonadotropin content, but at day 7 oxytocin halved pituitary gonadotropin content. The levels at day 0 were approximately 3 units/mg. of dried anterior pituitary weight in the controls, and 1.5 units/mg. in oxytocin-treated heifers. At day 4 the levels were about 10 in each, and at day 7 the levels had increased in

the controls to about 19, and oxytocin reduced them to about 8.

The most striking bit of evidence, I think, was when we did a correlation on the pituitary gonadotropin levels and the progesterone contents of the corpora. In control animals this correlation was large and negative, -0.75, but in the oxytocin-treated animals at day 7 the correlation was large, 0.96 and positive. This seemed to indicate at least in the oxytocin-treated animals, that pituitary level indicated plasma level and that it was the plasma levels of gonadotropin that were controlling and limiting corpus luteum function, as demonstrated by progesterone content or luteal weight. We believe that it is probably LH in this total gonadotropin fraction that is responsible for this result. We have not had very much success with a specific LH bioassay. The bioassay we used on these pituitaries was p32 uptake on the chick testis. It worked very well. We got quite good confidence limits and generally it was very satisfactory. I think this is fairly good evidence that LH is luteotropic in the bovine.

DR. HANSEL: May I add just two points to that statement? Perhaps the point about the urea-incubated HCG was not made clear. Incubation with urea is said to destroy the LH component of HCG and to destroy its ability to stimulate progesterone production of the corpora after the incubation. One other point that is perhaps worth presenting concerns the fact that one can produce larger than normal corpora by LH.

DR. DONALDSON: This shows a big CL that was grown with bovine LH. It weighed 9.5 grams at 7 days.

DR. MELAMPY: Was it solid?

DR. HANSEL: Yes. That one was.

Now, added to these data are those of Armstrong, Black, and Cone (Fed. Proc. 23: 462, 1964) which you see here in incomplete form. These are the results of their incubation studies in which they added the NIH bovine LH preparation to luteal tissue slices obtained from cows at various stages of the cycle: 0, 5, 6, and 9 et cetera. You will note that when they add LH they get a progesterone stimulation until day 17. Then, the stimulation cuts out, apparently rather abruptly, on day 18.

It seems to me, when you put together the in vivo results that Donaldson has talked about, with these in vitro results it is strongly indicated that LH is luteotropic. The only loop-hole that is left is that there could be some synergistic effect of FSH and LH as a result of the FSH contamination in the so-called purified LH preparation.

DR. NALBANDOV: I would just like to point out that I think these data are very nice but we should not confuse luteotropic effect, that is, the ability of the hormone to keep a corpus alive and functioning, with the ability of a hormone to make the corpus secrete progesterone in vitro. I think these effects are en-

tirely different and we may find that they depend on entirely different systems.

Would you like to comment on that?

DR. HANSEL: I am afraid I haven't much to add, but as I get the remark it is suggested that there is a difference between the ability of a compound to cause progesterone production in vitro, and its possible in vivo effect. To be sure, Armstrong's data are subject to this criticism, but the data that Donaldson cited are all in vivo data. So far as I know this is the only in vivo system for testing a luteotropic effect. It is the only way in which we can inhibit a corpus luteum, with the exception of estrogen injections, as was pointed out a while ago. These are in vivo results.

DR. NALBANDOV: In pituitary intact animals, however?

DR. HANSEL: Right.

DR. MELAMPY: Thank you. Any other questions?

DR. WILTBANK: I would like to mention that we have preliminary information at Fort Robinson on a limited number of animals. We are doing an experiment now in which we have been able to cause regression of the corpus luteum with around 640 mcg. of estrogen given daily. Then we have tried to reverse this effect with FSH and LH and we can get a reversal of the regression of the corpus luteum by giving these hormones, which, again, would indicate then that the LH is luteotropic and we can get it with just the LH alone.

DR. HANSEL: May I ask what kind of an LH preparation?

DR. WILTBANK: We are just using Armour's LH at the present time, so there was some FSH contamination in it.

DR. ANDERSON: In the hypophysectomized-hysterectomized gilt, corpora lutea are maintained from days 12 to 20 with Armour's LH. Corpora lutea are not maintained as well during this period in the hypophysectomized pig in which the uterus remains intact. The luteotropic effect of LH in the hypophysectomized-hysterectomized gilt is apparent, for in the absence of exogenous hormone support in these animals complete regression of corpora lutea and follicles occurs during this period.

DR. SPIES: I have two questions. One to Dr. Melampy and one to Dr. Wiltbank.

I will direct the first one to you, Jim. Since you have been able to produce regression of the fully formed corpus luteum in the cow with estrogen, would you say that this species differs from the pig and the ewe in that the cow corpus luteum depends on the pituitary throughout the cycle?

DR. WILTBANK: I think the indications that we have would say that this is true, that the bovine may be different than the ewe or the sow in this respect.

DR. SPIES: Dr. Melampy, I wish to ask you to comment on the statement that in the parabiotic rats you observed a shortening of the cycle. Was this observation based on the

vaginal cycle or histological study of the ovary?

DR. MELAMPY: We observed the cycling effect and also from the preparations we made histological observations on them, and the corpora lutea in some of these were in a state of regression.

Now there is one person here who has been working in this area and I would like to call on Dr. W. D. Foote because he has been doing some work on gonadotropic activity in the bovine pituitary and I think it is quite fitting that we should hear about this at this time.

DR. FOOTE: The work we have been doing at Nevada has not been directed toward the function of gonadotropins relative to their association with corpus luteum maintenance.

We have been trying to get basic information on what the pituitary is doing, in terms of FSH and LH activities in untreated animals. We have been working both with the beef heifer and to some extent the post-partum beef cow.

I think our results are interesting in view of the comments that were made regarding LH during the cycle. We have nothing that shows whether or not it has the luteotropic activity. However, the trend that we obtained for LH content of the pituitary gland is somewhat interesting. The LH content of pituitary glands taken at day 0, 2, 8, 16, and 19 of the estrual cycle showed the highest LH content to be present at day 16. The lowest content was at day 0. From day 0 there seemed to be a steady increase through day 8, up to the high level at

day 16. Then, for some reason, we found a decrease in LH activity between day 16 and day 19, which we could, I guess, consider to be related to the time the corpus luteum might normally start to regress and presumably decrease in progesterone production. We found a further decrease between day 19 and day 0. The FSH material is not quite complete, but it appears to follow somewhat the same trend during the cycle as the LH.

In the post-partum animals, we recovered pituitaries on day 5, day 17, and day 30, after calving, using only glands from cows which had not ovulated at the time of slaughter. The tendency here was for the lowest level of LH to be at day 5, an increase at day 17, and then a more marked increase between day 17 and day 30. This indicates, I suppose, that during this recovery period, or whatever the nature is of the post-partum anestrual period, the cow is beginning to build up again the level of LH. This, of course, refers to pituitary content. It does not tell us what is actually present in the blood. Here, again, the FSH picture is not complete, but it does not seem to follow the LH trend as it did in the cycling heifers. In fact, our results suggest that an inverse relationship may exist between LH and FSH levels at this reproductive stage.

DR. MELAMPY: Thank you very much. I want to thank Dr. Anderson, Dr. Day, and Dr. Spies for participating and also the members in attendance at the symposium.

We stand adjourned until tomorrow morning.